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Page) of 1

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Prev Page Next Page Go to Doc#

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## (19) United States

# (12) Patent Application Publication (10) Pub. No.: US 2004/0029238 A1

Rajgarhia et al.

(43) Pub. Date: Feb. 12, 2004

#### (54) METHODS AND MATERIALS FOR THE SYNTHESIS OF ORGANIC PRODUCTS

(76) Inventors: Vineet Rajgarhia, Minnetonka, MN (US); Vassily Hatzimanikatis, Minneapolis, MN (US); Stacey Olson, Minneapolis, MN (US); Ting Carlson, Dayton, OH (US); John N. Starr, Chaska, MN (US); Jeffrey J. Kolstad, Wayzata, MN (US); Aharon Eyal,

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Jerusalem (IL)

(21) Appl. No.:

10/287,564

(22) Filed:

Nov. 4, 2002

### Related U.S. Application Data

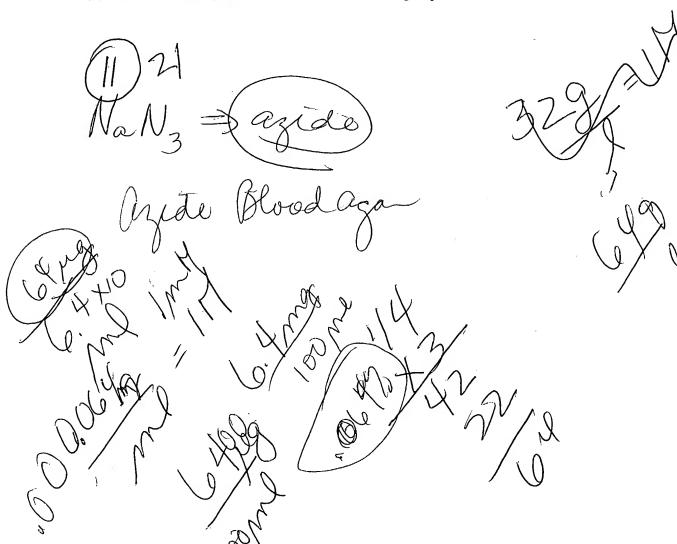
(63) Continuation of application No. 09/574,873, filed on May 19, 2000, now Pat. No. 6,485,947, which is a continuation-in-part of application No. 09/316,490, filed on May 21, 1999, now abandoned.

#### **Publication Classification**

Int. Cl.<sup>7</sup> ...... C12P 7/56; C12N 1/20 

#### (57)**ABSTRACT**

The invention provides methods and materials related to the production of organic products. Specifically, the invention provides various recombinant yeast cells, methods for culturing yeast cells, methods for making yeast cells, nucleic acid constructs, and methods and materials for producing various organic products.



<u>Previous Doc</u> <u>Next Doc</u> <u>Go to Doc#</u> First Hit

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L6: Entry 6 of 164

File: PGPB

Feb 12, 2004

DOCUMENT-IDENTIFIER: US 20040029238 A1

TITLE: Methods and materials for the synthesis of organic products

#### Detail Description Paragraph:

[0109] In general, culture medium containing an inhibitor of cellular respiration (e.g., antimycin A, cyanide, and <u>azide</u>) can reduce cellular respiration, while the absence of such inhibitors can promote cellular respiration. Likewise, <u>anaerobic</u> culture conditions can reduce cellular respiration, while aerobic culture conditions can promote cellular respiration. An aerobic condition is any condition where oxygen is introduced or occurs naturally and serves as a substrate for the respiratory pathway. Generally, the term "aerobic" refers to a culture condition in which the culture media is maintained under an air flow of at least 0.1 VVM (volume air/volume liquid/minute) (e.g., greater than 0.2, 0.3, 0.4, 0.5, 1.0, 1.5 or 2.0 VVM). If a gas other than air is used then the nominal VVM is adjusted to an air equivalent based on oxygen content of the gas. Alternately, "aerobic" can be defined as a culture media which has a dissolved oxygen content of at least 2 percent (e.g., at least 5, 10, 20, 30, 40, 50, 60, 75 or 80 percent) relative to the amount present at saturated conditions with air at atmosphereic pressure.

Previous Doc Next Doc Go to Doc#

<u>Previous Doc</u> <u>Next Doc</u> <u>Go to Doc#</u> <u>First Hit</u>

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L6: Entry 10 of 164

File: PGPB

Sep 25, 2003

DOCUMENT-IDENTIFIER: US 20030180272 A1

TITLE: Probiotics in primary prevention of atopic diseases

#### Detail Description Paragraph:

[0042] A faecal sample from the infant was taken either by nursing staff at scheduled visit or immediately prior to it by parents. In the latter case, the sample was stored at 4.degree. C. and delivered to the hospital within 24 hours for immediate cultivation. A stool sample was obtained from 71 infants at the age of 20 days (18 to 21 days) and from 69 at the age of 14 weeks (13 to 14 weeks); mean (95% CI). The rest of the sample was immediately frozen and stored at -20.degree. C. until analysed by GLC and FISH. No quantitative culture methods were employed. The bacteria were cultured on 6 different freshly prepared media, i.e. Blood Agar (Pronadisa, Madrid, Spain) for gram-negative rods; agar (Leiras, Turku, Finland) supplemented with Mycological Peptone (Oxoid, Basingstoke, United Kingdom) and glucose for yeasts and fungi; Bile Eskulin Azide Agar (Difco, Detroit, USA) for enterococci; Blood Agar (Pronadisa) supplemented with glucose, yeast extract (LAB M, Bury, United Kingdom), L-cysteine HCl (Merck, Darmstadt, Germany), metadion (Merck) and neomycin sulfate (Sigma, St. Louis, USA) for anaerobes; Clostridium difficile Agar (Oxoid) supplemented with hemin (Sigma), neutralred (Merck), D-Cycloserine (Sigma), egg and Cefoxitin (MSD, Haarlem, the Netherlands) for Clostridium difficile; and Rogosa SL agar (Difco) for Lactobacillus-like bacteria. The first three media were incubated aerobically and the last three anaerobically at 35.degree. C. for 48 h. Subsequently, identification of different species was made according to their growth on selective media, colonies, color and cell morphology.

Previous Doc Next Doc Go to Doc#

#### First Hit

L4: Entry 1 of 8

File: PGPB

Jul 24, 2003

PGPUB-DOCUMENT-NUMBER: 20030138867

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030138867 A1

TITLE: Medium composition, method and device for selectively enhancing the isolation isolation of <a href="mailto:anaerobic">anaerobic</a> microorganisms contained in a mixed sample with facultative microorganisms

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

NAME CITY STATE COUNTRY RULE-47

Copeland, James C. Ashland OH US Myers, Kathy J. Mansfield OH US

APPL-NO: 10/ 007739 [PALM]
DATE FILED: November 8, 2001

RELATED-US-APPL-DATA:

Application is a non-provisional-of-provisional application 60/246872, filed November 8, 2000,

INT-CL: [07] G01 N 33/554, G01 N 33/569, C12 Q 1/04

US-CL-PUBLISHED: 435/7.32 US-CL-CURRENT: 435/7.32

REPRESENTATIVE-FIGURES: NONE

#### ABSTRACT:

The present invention is directed to a <u>medium</u>, <u>broth or agar</u>, and a method of utilizing the same, in order to isolate and/or identify <u>anaerobes</u> from a mixed sample that contains facultative microorganisms. The <u>medium</u> contains an inhibitor of the electron transport system, such as a salt of <u>azide</u> (N.sub.3.sup.-), cyanide (CN.sup.-) or related compounds. These inhibitors are present in an amount sufficient to limit the growth of facultative microorganisms under <u>anaerobic</u> conditions while not inhibiting the growth of the <u>anaerobe</u> microorganisms. Preferably, the inhibitor is present in the amount of from about 0.1 mg/ml to about 1.0 mg/ml in broth medium, and from about 0.01 mg/ml to 1.0 mg/ml in agar medium.

[0001] The present application claims the benefit of priority to U.S. Provisional Application Serial No. 60/246,872 filed on Nov. 8, 2000.

### First Hit Fwd Refs

L6: Entry 31 of 164

File: USPT

Nov 26, 2002

DOCUMENT-IDENTIFIER: US 6485947 B1

TITLE: Production of lactate using crabtree negative organisms in varying culture conditions

#### Detailed Description Text (51):

In general, culture medium containing an inhibitor of cellular respiration (e.g., antimycin A, cyanide, and <u>azide</u>) can reduce cellular respiration, while the absence of such inhibitors can promote cellular respiration. Likewise, <u>anaerobic</u> culture conditions can reduce cellular respiration, while aerobic culture conditions can promote cellular respiration. An aerobic condition is any condition where oxygen is introduced or occurs naturally and serves as a substrate for the respiratory pathway. Generally, the term "aerobic" refers to a culture condition in which the culture media is maintained under an air flow of at least 0.1 VVM (volume air/volume liquid/minute) (e.g., greater than 0.2, 0.3, 0.4, 0.5, 1.0, 1.5 or 2.0 VVM). If a gas other than air is used then the nominal VVM is adjusted to an air equivalent based on oxygen content of the gas. Alternately, "aerobic" can be defined as a culture media which has a dissolved oxygen content of at least 2 percent (e.g., at least 5, 10, 20, 30, 40, 50, 60, 75 or 80 percent) relative to the amount present at saturated conditions with air at atmosphereic pressure.